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TITLE: Targeted Prevention or Treatment of Bacterial Biofilm Infections of Severe Burns and Wounds

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Final Report
DR080371: Targeted Prevention or Treatment of Bacterial Biofilm Infections of Severe Burns and Wounds

Introduction: Persistent infection of severe wounds, and burns in particular, represents a significant cause of deployment-related morbidity and mortality. Inability to successfully treat wound and burn infections relates to the capacity of the bacteria to form a **biofilm**[1]. In patients with severe burns, 75% of deaths will occur from sepsis or infectious complications, with *P. aeruginosa* accounting for over half of all burn infections. In the setting of thermal injury, an intense inflammatory response is universally present, culminating in massive recruitment of **neutrophils** to the tissue[1]. In both thermal and reperfusion injury, vascular spasm and impaired blood flow to the site is followed by reperfusion and vascular leak. Importantly, this tissue injury uncovers **self antigens** on the nonmuscle myosin heavy chain II (NMHC-II) that are recognized by a specific natural IgM subclass (IgM^{CM-22}), as an early response of the innate immunity (**Figure 1**). Binding of IgM^{CM-22} to NMHC-II triggers the complement cascade, and the subsequent recruitment of neutrophils to the site of injury[2]. Systemic or local administration of a 12-mer synthetic peptide (N2) analogous to NMHC-II is capable of binding and competitively inhibiting IgM^{CM-22}, thus greatly reducing inflammation at the site of the wound[2]. Excessive neutrophil accumulation, combined with impaired clearance of the dead and dying cells, is clearly linked to tissue damage. However, recent reports have demonstrated that neutrophil products can accelerate *P. aeruginosa* biofilm formation[3]. As neutrophils undergo necrosis, long strands of DNA and F-actin are released into the inflammatory milieu, and polymerize through covalent attraction. Recently it was reported that *P. aeruginosa* can exploit the neutrophil-rich environment by utilizing these polymers as a scaffolding, significantly enhancing early biofilm formation[3]. A strategy to limit local neutrophil influx, without systemically suppressing the immune system, represent as novel approach to both **prevent** biofilm formation, and possibly as an adjuvant to medically **treat** an early biofilm infection. Concurrent with reduced tissue inflammation, a different approach to antibiotic therapy is proposed. Macrolide antibitoics such as azithromycin have now been demonstrated to have a potent antimicrobial effect when *P. aeruginosa* is allowed to form a biofilm[4]. Azithromycin has excellent penetration into tissues, as well as potent intrinsic anti-inflammatory properties independent of its function as an antimicrobial. Therefore, we will test the effect of a dual therapeutic approach combining a targeted anti-inflammatory with a biofilm specific antibiotic to significantly reduce local and systemic infection associated with serious burns and wounds.

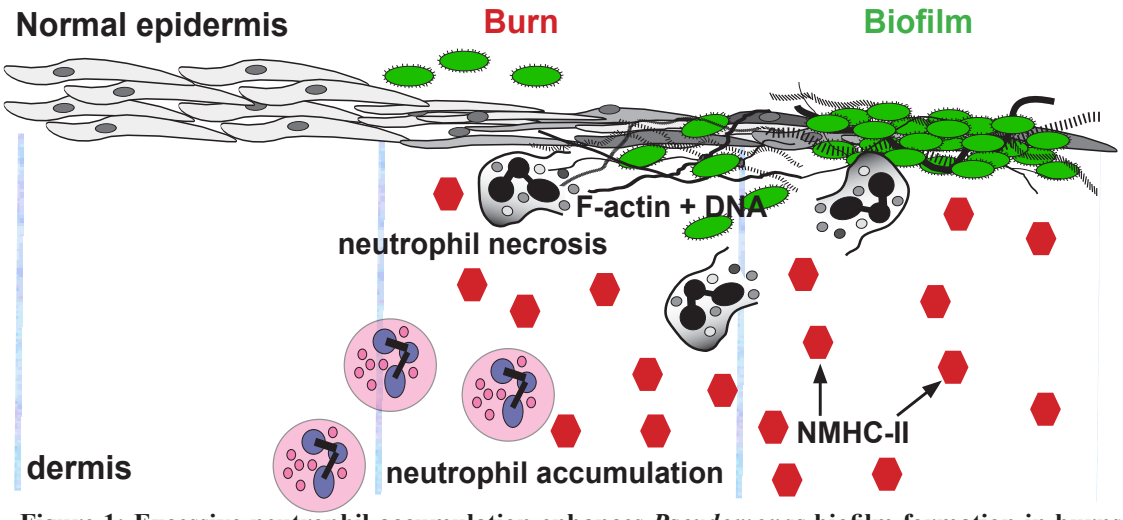

Figure 1: Excessive neutrophil accumulation enhances *Pseudomonas* biofilm formation in burns and wounds. Tissue damage exposes the self-antigen NMHC-II (red polygons), which is bound by IgM^{CM-22}, resulting in activation of the complement cascade. The ensuing recruitment of massive quantities of neutrophils overwhelms mechanisms to clear dying cells from the tissues. DNA and F-actin released from necrotic neutrophils form polymers, which serve as a scaffolding for *P. aeruginosa* and accelerates the formation of biofilms.

Body:
Over the 18 months of this proposal, we have completed all of the stated Aims within the approved Statement of Work. Results of the experiments have supported the validity of the novel use of a macrolide antibiotic (azithromycin) in the context of a *P. aeruginosa* post-burn infection. However, we have determined that combining the anti-inflammatory strategy with antibiotics was unexpectedly injurious. Complete findings are presented below, and organized as they relate to the proposed Tasks and Milestones within the Statement of Work, which are unchanged from the original application.

Milestone 1: Synthesis of N2 peptide and 12-mer scrambled peptide (control). (**Timeframe 1 month**)

Results: The N2 peptide and 12-mer scrambled control peptide were successfully synthesized in sufficient quantities to complete the entire proposal.

Milestone 2: Institutional Review of modification to existing animal protocols. (**Timeframe 1-2 month**).

Results: All required amendments to our existing protocol (AS2751_04_10) were approved by the National Jewish Health IACUC in December 2009, and the corresponding approval by the ACURO was completed in January 2010. In April 2010, the original protocol was required to undergo a routine Triennial Review, which mandated the entire protocol be rewritten and re-reviewed. The new protocol (AS2751_03_13) was approved by the NJH IACUC in April 2010. As a result of this Triennial Review, the protocol was then required to be re-reviewed by ACURO. The current protocol received re-approval in June 2010. Given the sensitive nature of inflicting a burn, followed by infection with *P. aeruginosa*, we were aware that the procedure would received considerable scrutiny. Nonetheless, the entire time involved in meeting regulatory requirements resulted in some delays in meeting proposed milestones. All proposed experiments have been approved by the NJH IACUC and the ACURO, and no further regulatory reviews were required before the completion of the Project.

Milestone 3: Test the effect of pre-administration of N2 peptide (i.v.) and/or (topical) to reduce post-burn *P. aeruginosa* wound infection. (**Timeframe 2-6 months**)

Results: The purpose of these proposed experiments was to define the conditions by which N2 peptide evokes the greatest anti-inflammatory effect. Comparisons were made between i.v. and topical administration, as well as the potential for an additive effect of the two administration methods combined. **The goal** was determination of the optimal method of N2 administration associated with suppression of inflammation of neutrophil accumulation to the skin (assayed by MPO and histology), with the most effective dose and delivery of N2 peptide to be utilized in subsequent tasks of this proposal.

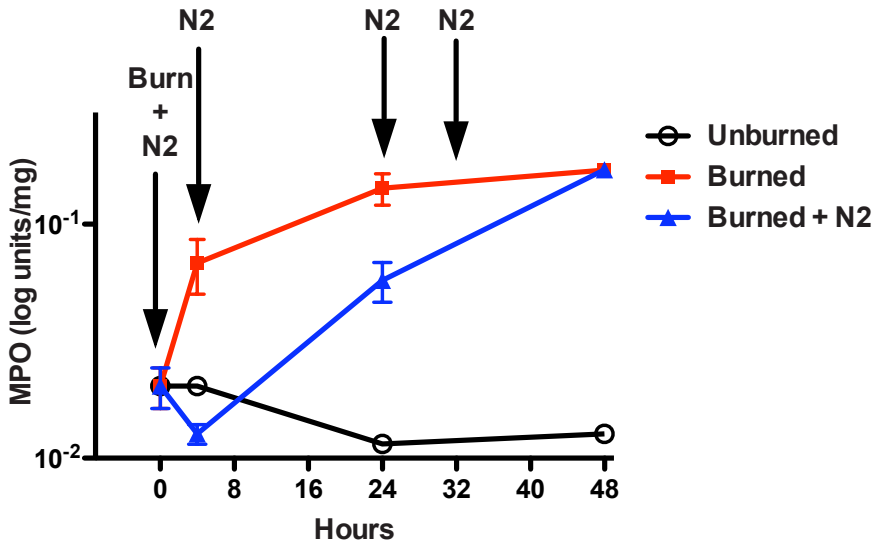

Figure 2: Effect of N2 peptide in reducing neutrophil accumulation to the skin following thermal injury. Animals burned at time 0 received N2 peptide administered IV through tail vein injection and topically at the site of the burn. The agent was administered at the times depicted by arrows. Neutrophil accumulation was assessed by MPO activity in skin homogenates at the burn site. After 4 hours, the N2 peptide dramatically reduced neutrophil recruitment to the burn, but this effect was lost over time, despite repeated administration of the compound. The effect of the N2 administration over the entire 48-hour period studied was significant, with p=0.0023 by two-way ANOVA.

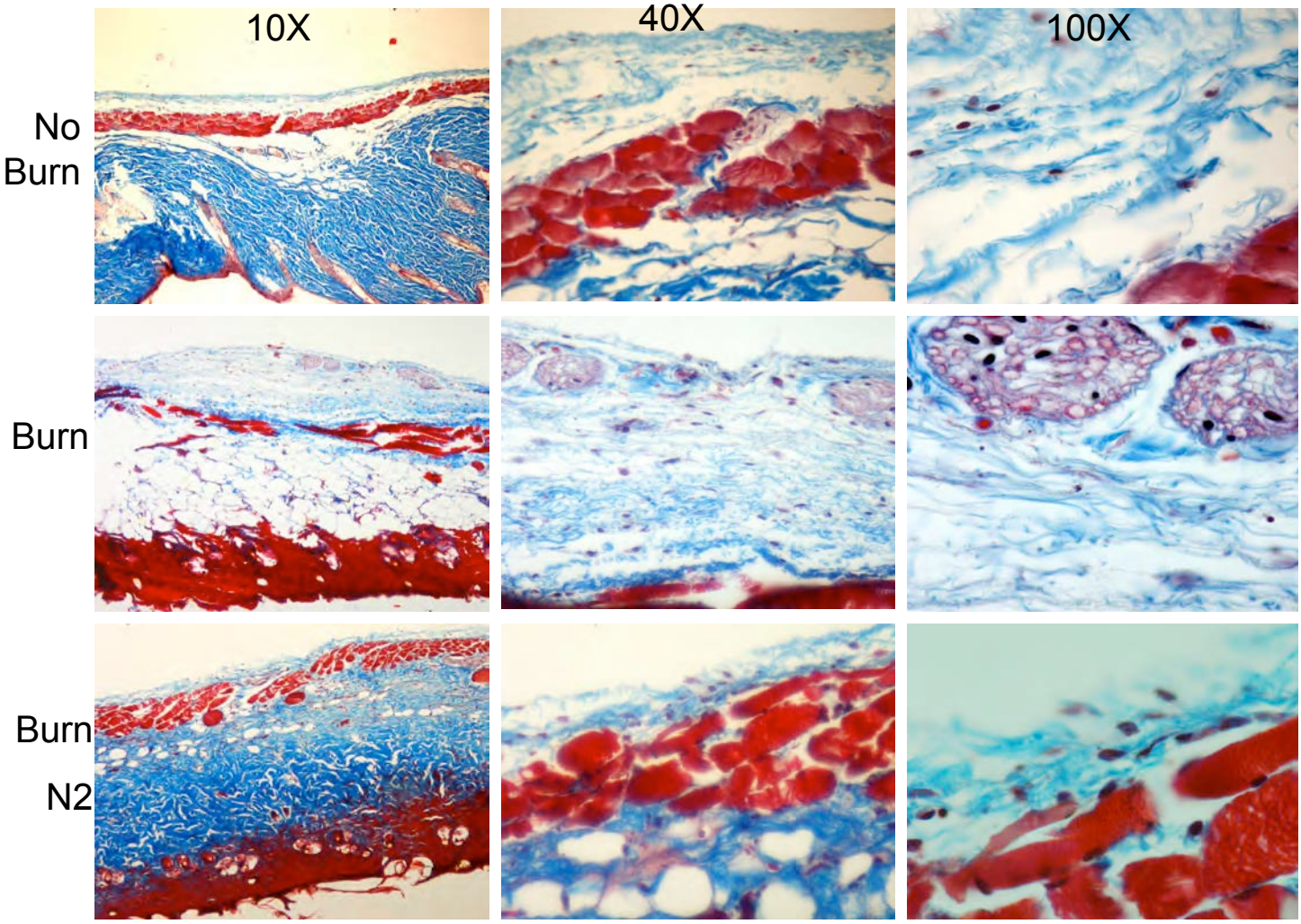

Figure 3: Effect of N2 peptide in reducing subcutaneous inflammation following thermal injury. Animals burned at time 0 received N2 peptide administered IV through tail vein injection and topically at the site of the burn. Photomicrographs depict histological changes 4 hours following the burn for a representative animal that was shaved but not burned (top row), burned but received no compound (middle row), or burned and received N2 at the time of the burn. Magnification is as labeled for each column. Results validate the findings of the MPO assay (**Fig. 2**), as neutrophil accumulation is not present in the N2 treated animals. In addition, these results indicate that N2 also results in nearly complete elimination of tissue edema and other morphologic changes of the burn, suggesting that these early events are neutrophil-mediated. Figures representative of 3 animals in each group, with areas of injury that were typical.

N2 peptide works to bind and competitively inhibit $IgM^{CM\text{-}22}$ that recognizes self antigens on the nonmuscle myosin heavy chain II (NMHC-II) that are exposed at the time of injury. In pilot experiments, we determined that administration of the N2 peptide prior to the burn afforded no protective advantage, as the injury had not yet occurred, and $IgM^{CM\text{-}22}$ was not yet present. Thus, N2 peptide can be administered at the time of the injury with maximal effectiveness, which would actually represent a more plausible use of the agent in the setting of combat.

As proposed, a series of time courses and dose response assessments of I.V. and/or topical application of N2 were conducted. We determined that when administered in the setting of the thermal injury alone, the optimal delivery of N2 was the combination of both the I.V. (200ul of 40uM solution) and topical administration (200ul of 40uM). Best results were seen when the topical N2 was combined with DMSO to allow for rapid subcutaneous absorption. With these doses, the animals tolerate the agent without apparent toxicity. However, at higher doses we observed that the ears of the animals underwent necrotic changes. In response to the thermal injury alone, the anti-neutrophil effect of N2 is profound at 4 hours, but even with repeated dosing this effect is no longer present by 48 hours (**Figure 2**). However, this early anti-neutrophil effect is also associated with a broader anti-inflammatory effect at 4 hours, which is sufficient to prevent nearly all evidence of thermal injury by histology (**Figure 3**). By 48 hours, animals that were burned but did not receive N2 demonstrated significant tissue injury, ulceration and eschar formation (**Figure 4**). In contrast the animals that had received N2 at the time of the burn demonstrated

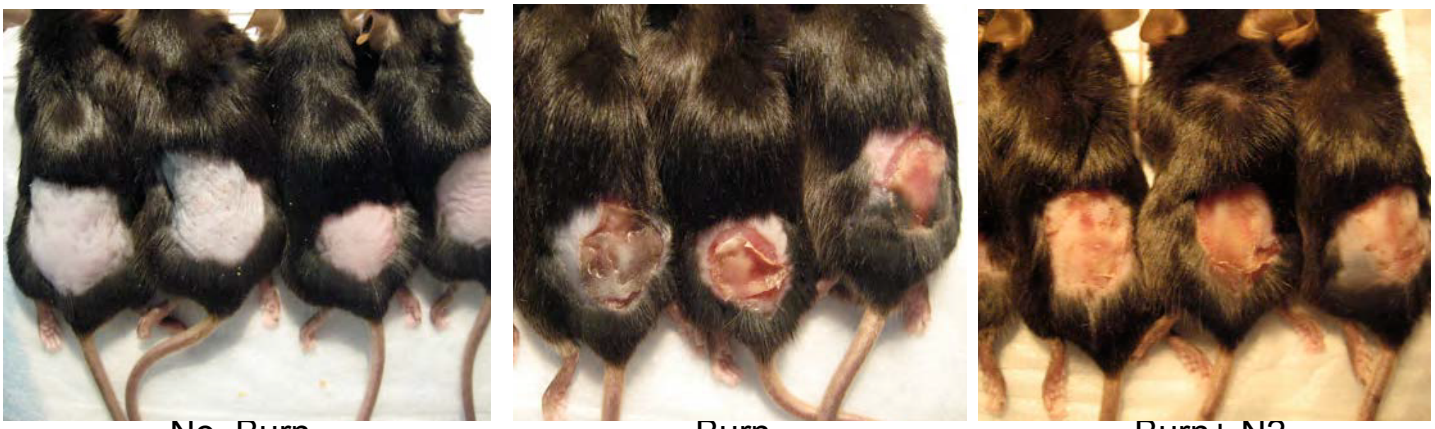

Figure 4: Effect of N2 peptide in reducing gross tissue damage following thermal injury. Animals burned at time 0 received N2 peptide administered IV through tail vein injection and topically at the site of the burn. Photographs demonstrate gross tissue injury 48 hours the burn for cohorts of four animals that were shaved but not burned (left), burned but received no compound (middle), or burned and received N2 at the time of the burn (right). While significant ulceration and eschar formation is evident in the burned animals, the extent of gross injury is clearly less in the group receiving N2, despite the relatively short-duration of anti-neutrophil effects at the site of the injury.

much less severe gross tissue damage (**Figure 4**). Together, the results of these experiments indicated that N2 could be successfully administered at the time of the burn, and that a single administration is sufficient to evoke a profound anti-neutrophil effect early after injury, that results in significant reduction in tissue damage at later timepoints.

When the experimental model was expanded to include both a thermal injury followed by cutaneous infection with *P. aeruginosa*, the administration of N2 also resulted in a significant reduction in inflammation at early timepoints (**Figure 5**). However, as seen in the absence of infection, this effect is not present at a later timepoint.

Of greatest importance to this proposal is the capacity of N2-mediated neutrophil inhibition to afford protection against *P. aeruginosa* infection. At the 8 hour timepoint, we found a dramatic reduction in P. aeruginosa infection in the skin (**Figure 6**). At the 24 hour timepoint, partial protection remained, which generally correlates to the decreasing anti-inflammatory effect of the N2 peptide. However, overall the effect was significant (p=0.01 by two-way ANOVA). With later time points, the protective benefit was no longer present (not shown). Together, these results support the validity of our Central Hypothesis, and represent completion of Milestone 3.

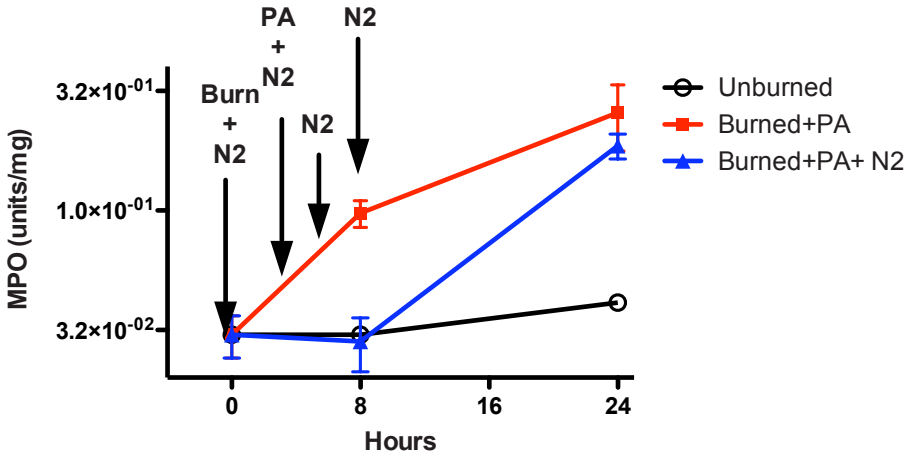

Figure 5: Effect of N2 peptide in reducing neutrophil accumulation to the skin following thermal injury associated with *P. aeruginosa* infection. Animals burned at time 0 received N2 peptide administered IV through tail vein injection and topically at the site of the burn. Two hours after the thermal injury, the site was infected with *P. aeruginosa*, and N2 was administered again. The agent was given twice more in the first 8 hours, as depicted by arrows. Neutrophil accumulation was assessed by MPO activity in skin homogenates at the burn site. As seen in the absence of infection, the N2 peptide dramatically reduced neutrophil recruitment to the burn, but this effect was lost over time, despite repeated administration of the compound. The effect of the N2 administration over the time studied was significant, with p=0.004 by two-way ANOVA.

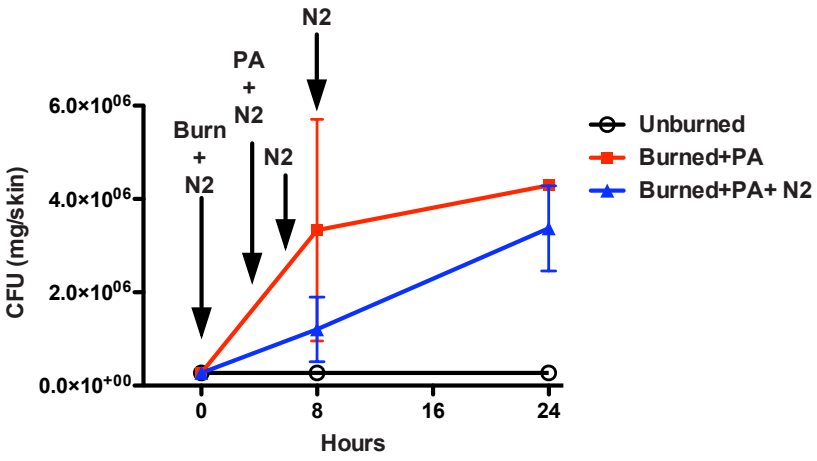

Figure 6: Effect of N2 peptide in reducing bacterial burden associated with *P. aeruginosa* infection in the burn model. Quantity of bacteria present under the condition described for **Figure 5** was analyzed by standard methods. Associated with decreased neutrophil accumulation to the dermis was a decrease in burden of *P. aeruginosa*. This effect was greatest at the earlier timepoint, corresponding to the pattern of neutrophil influx. Effect of treatment over the time studied was significant, with p=0.01 by two-way ANOVA.

Milestone 4: Determination if azithromycin reduces wound infection beyond what is achieved by N2 peptide alone.

Results: A maximum dose of AZM for the mouse model was chosen from the literature. Based on the excellent tissue penetration and pharmokinetics of the antibiotic, a single dose of AZM was tested. Unexpectedly, we found that the combination of AZM with N2 resulted in dramatic worsening of *P. aeruginosa* burn infections. While the model was not designed to test mortality, overall degree of illness is reflected in weight loss. Mice were shaved, and 3 days later burned and infected with *P. aeruginosa*. Animals that received the combination of AZM with N2 consistently demonstrated the greatest weight loss (**Figure 7**). Corresponding with greatest weight loss, the greatest burden of skin infection at 72 hours was also found in animals treated with AZM and N2 (**Figure 8**). In addition, systemic infection, as measured by *P. aeruginosa* recovered from the lungs and spleen was also greatest in the animals treated with the combination (**Figure 8**).

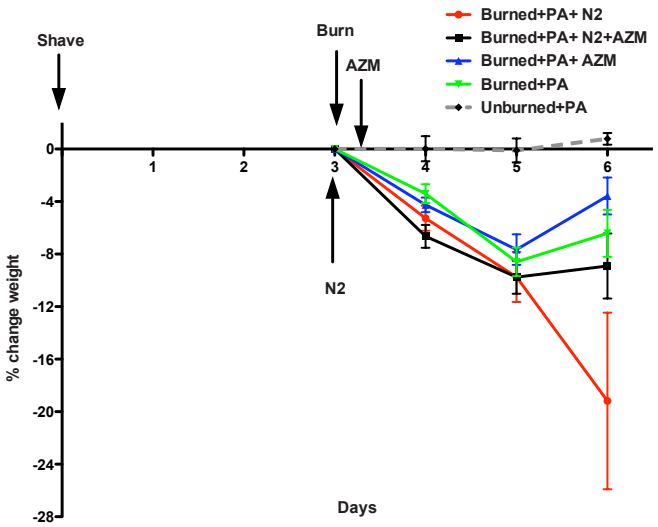

Figure 7: Effect of N2 peptide combined with azithromycin on animal weight loss. The combination of anti-inflammatory treatment with the macrolide antibiotic consistently accelerated weight loss following burn and *P. aeurginosa* infection in the murine thermal injury model. Plot depicts percent change in weights, from 5 separate trials, with 3-5 mice per time point per condition per trial, with approximately 125 total animals.

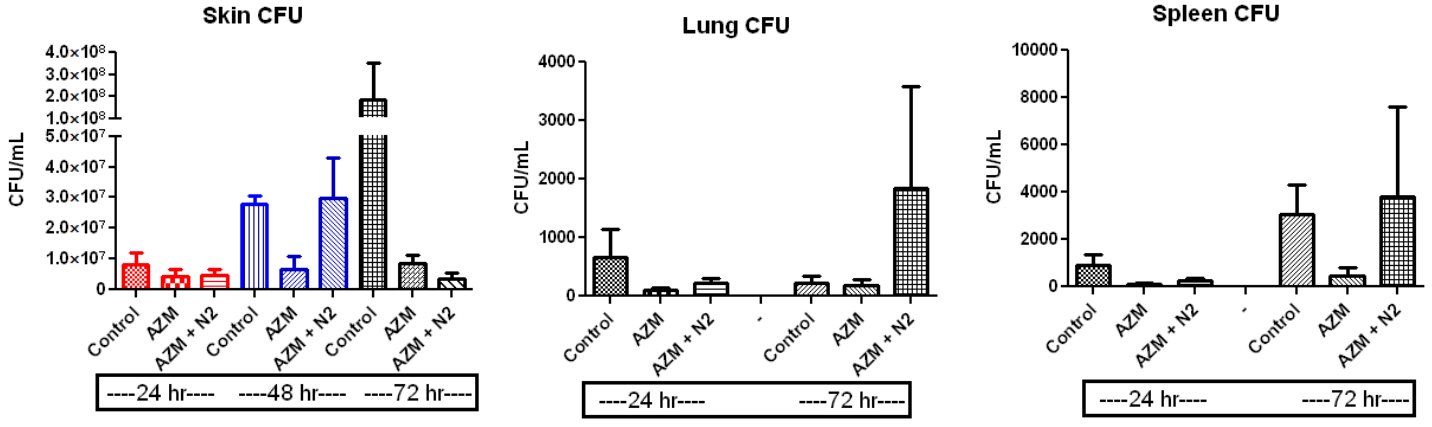

Figure 8: Effect of N2 peptide combined with azithromycin to reduce post-burn *P. aeruginosa* wound infection. The combination of anti-inflammatory treatment with the macrolide antibiotic consistently worsened the burden of *P. aeurginosa* infection in the murine thermal injury model. The effect on the skin (Left Panel) was also reflected in worse systemic infection in both the lung (Middle Panel) and the spleen (Right Panel). The same result was observed whether AZM was administrated before or after the thermal injury.

Milestone 5: Prove or disprove the utility of the N2 peptide as an effective local anti-inflammatory in the setting of an ongoing wound infection (assayed by MPO and histology).

Results: Five time courses of N2 peptide administration demonstrated that the molecule only has anti-inflammatory properties when administrated within hours of the thermal injury (**Figure 5**). We found that pre-administration does not enhance this effect, and as discussed above, serial administration after the initial burn does not produce a greater effect than a single dose.

Milestone 6: Determine if the N2 peptide combined with azithromycin reduces the bacterial burden of an ongoing wound infection (assayed by cfu's per gram of tissue).

Results: As demonstrated in **Figures 7 and 8**, there was no evidence of benefit from the combination of treatments, even when the biofilm infection was very light. Given the lack of N2 effect following the first few hours post-injury, we determined that further trials with more established (more severe) infections was unlikely to result in improvement. Together, we feel that Milestones 4-6 conclusively demonstrate that while N2 has been previously shown to have benefit in accelerating healing of non-infected burns, it has little practical application in the setting of a post-thermal injury infection. However, control animals treated with azithromycin in the absence of N2 did demonstrate improved response to injury. The benefit of azithromycin for treatment of *P. aeruginosa* burn infections has never been reported. However, this is analogous to the significant benefit that has been seen in Cystic Fibrosis patients chronically infected with P. aeruginosa.

Milestone 7: Determine if N2 peptide with azithromycin enhances the efficacy of tobramycin (assayed by cfu's per gram of tissue).

Results: Given the apparent benefit of azithromycin, and lack of sustained benefit from N2, we tested the utility of combining azithromycin with conventional antibiotics that are typically used for the treatment of serious *P.*

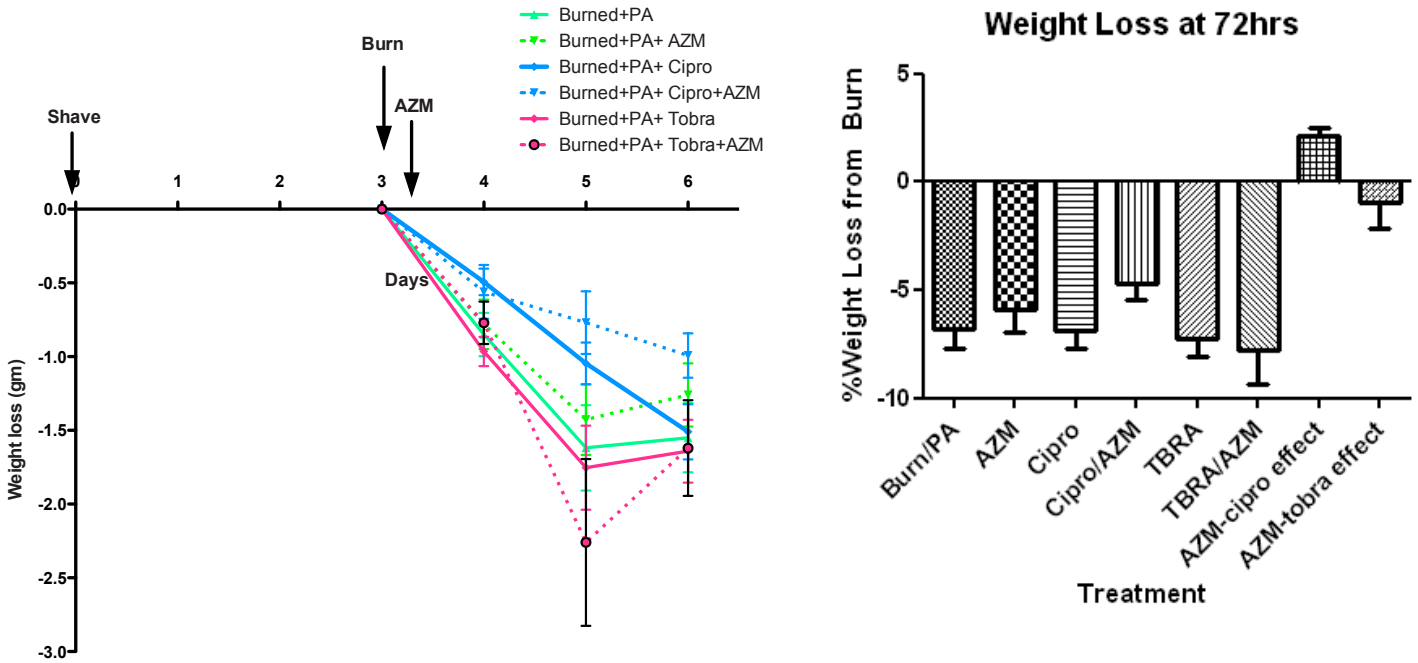

Figure 9: Effect of azithromycin combined with anti-*Pseudomonal* antibiotics on animal weight loss. The combination of azithromycin with tobramycin resulted in accelerated weight loss. However, azithromycin as a single agent, or azithromycin combined with ciprofloxacin demonstrated a reduced rate of weight loss following burn and *P. aeurginosa* infection in the murine thermal injury model. **Left Panel**: Percent change in weights, from 4 separate trials, with 3-5 mice per time point per condition per trial, with approximately 100 total animals. **Right Panel:** Percent weight change at 72 hours, with the relative change induced by azithromycin on either ciprofloxacin or tobramycin administration plotted on the far right columns.

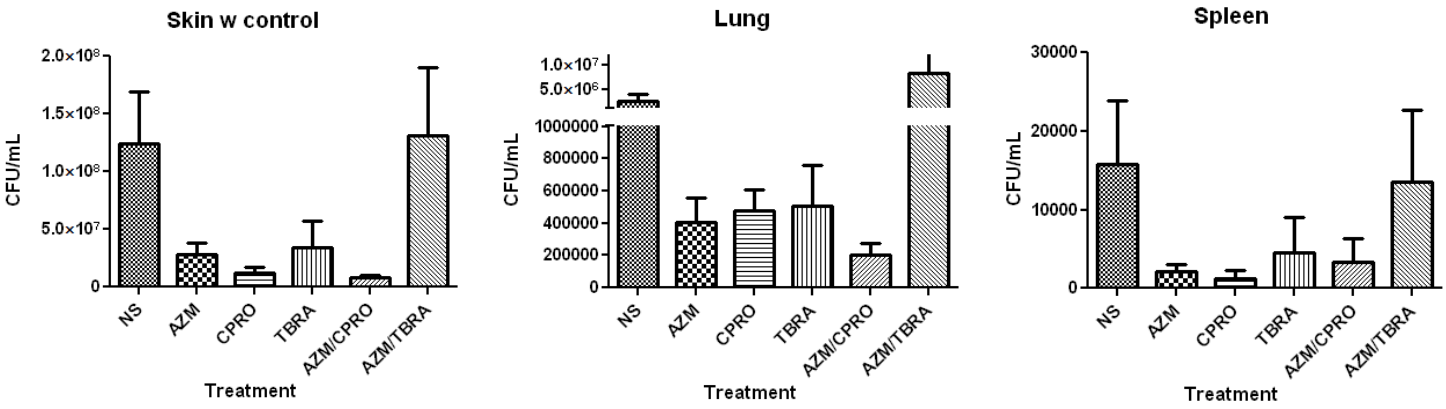

Figure 10: Effect of azithromycin combined with anti-*Pseudomonal* antibiotics on infection. The combination of azithromycin with tobramycin resulted in worsening of infection. However, azithromycin as a single agent, or azithromycin combined with ciprofloxacin demonstrated a reduced local and systemic infection following burn and *P. aeurginosa* infection in the murine thermal injury model. The effect on the skin (Left Panel) was also reflected in worse systemic infection in both the lung (Middle Panel) and the spleen (Right Panel). Infection was analyzed at 72 hours.

aeruginosa infections. As proposed, we tested tobramycin (dosed once daily), and in addition we tested the fluoroquinolone ciprofloxacin (dosed twice daily). Primary analysis was change in weight (**Figure 9**), burden of infection (cfu's) in skin, lung and spleen (**Figures 10-11**), and skin histology (**Figure 12**). We found that the combination of azithromycin and ciprofloxacin had an additive benefit compared to each agent alone. Unexpectedly, we found the combination of azithromycin with tobramycin was actually antagonistic, with increased skin and systemic infection (**Figures 10-11**), and accelerated weight loss (**Figure 9**). This represents a potentially important finding, as if results of this work lead ultimately to the routine use of azithromycin in the setting of thermal injury, the potential for either synergy or antagonism with standard antibiotics will need to be analyzed thoroughly. Recently, there has been an *in vitro* report that demonstrated an antagonistic decrease in antibiotic-induced killing of CF strains of *P. aeruginosa* by the combination of azithromycin and tobramycin[6]. Our findings are the first demonstration of this effect *in vivo*.

Milestone 8: Preparation and initial submission of the manuscript.

Results: We have initiated work on the first of two manuscripts that will describe this work.

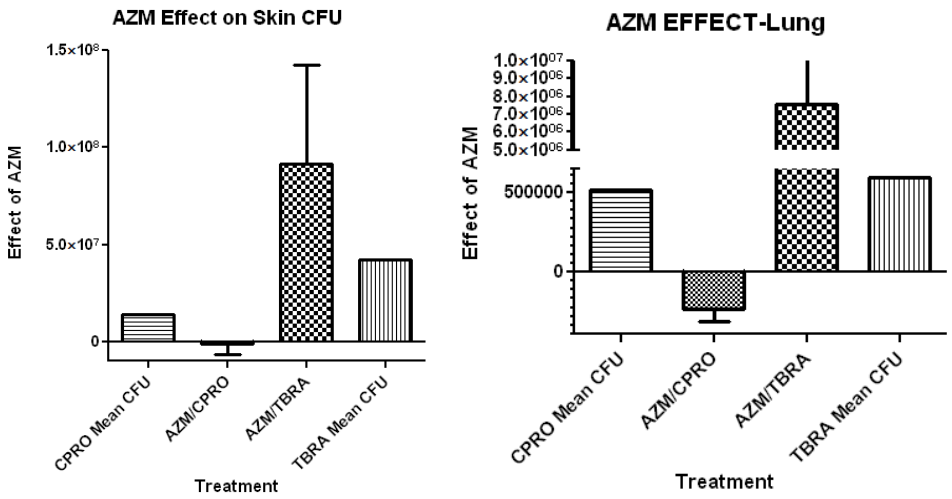

Figure 11: Effect of azithromycin combined with anti-*Pseudomonal* antibiotics on infection. Data from Figure 10 replotted to demonstrate the divergent effect of azithromycin with tobramycin compared to azithromycin combined with ciprofloxacin on local and systemic infection following burn and *P. aeurginosa* infection in the murine thermal injury model. The effect on the skin (Left Panel) was also reflected in worse systemic infection in both the lung (Right Panel) and the spleen (not shown). Infection was analyzed at 72 hours.

Azithromycin with tobramycin **Azithromycin with ciprofloxacin**

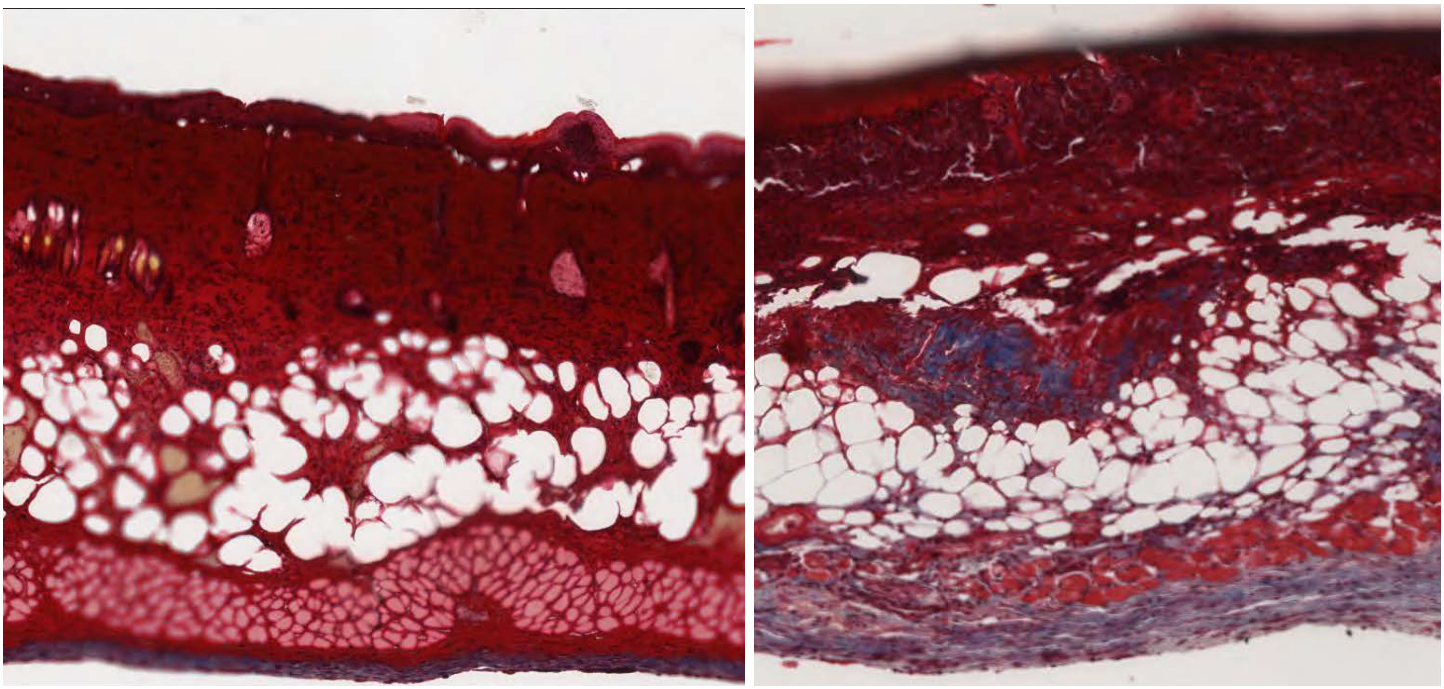

Figure 12: Effect of azithromycin combined with anti-*Pseudomonal* antibiotics on infection. Representative pathological samples of skin from animals treated with azithromycin plus tobramycin (Left Panel) compared to azithromycin combined with ciprofloxacin (Right Panel) following burn and *P. aeurginosa* infection in the murine thermal injury model. The greater severity of tissue injury is apparent in the AZM/Tobra samples, with loss of epidermal structure.

Key Research Accomplishments

- Synthesis of the N2 peptide and scrambled 12-mer control
- Identification of the optimal dose and delivery method of the N2 peptide
- Definition of the maximum duration of effect of N2 on reducing neutrophil accumulation to the skin following thermal injury
- Identification of broader anti-inflammatory effects in reducing tissue swelling and injury following thermal injury
- Demonstration of longer-term effects in reducing gross tissue damage following thermal injury
- Demonstration of the effectiveness of N2 in reducing neutrophil accumulation in the setting of post-burn *P. aeruginosa* infection
- Demonstration of the association between decreased neutrophil-mediated inflammation and decreased infectious burden by *P. aeruginosa*.
- Demonstration of a lack of benefit evoked by N2 in reducing *P. aeruginosa* infection as a single agent, under the conditions tested.
- Demonstration of an antagonistic interaction between N2 and azithromycin in the post-burn *P. aeruginosa* infection model.
- Demonstration of the benefit of azithromycin as a single agent to reduce infection and weight loss in the in the post-burn *P. aeruginosa* infection model.
- Demonstration of an additive benefit of the combination of azithromycin with ciprofloxacin to reduce infection and weight loss in the in the post-burn *P. aeruginosa* infection model.
- Demonstration of an apparent antagonism from the combination of azithromycin with tobramycin under the conditions studied.

Reportable Outcomes

1. Our findings are the first to test the efficacy of the N2 peptide in a burn that is complicated by infection, and indicate that N2 does not have benefit in this common scenario.
2. Our findings are the first to identify a benefit to azithromycin administration in the setting of a burn and a post-burn *P. aeruginosa* infection. This simple therapy, which could be administered as a once daily oral dose, could potentially change the approach to treatment of deployment-related burns and injuries that have a high likelihood of subsequent infection with *P. aeruginosa*. As this drug is an FDA-approved antibiotic that is widely used, a clinical trial to validate this result could be initiated without further pre-clinical testing.
3. Our findings are the first in vivo demonstration of potential antagonism between azithromycin and tobramycin in the setting of a ***P. aeruginosa*** biofilm infection. This supports recent in vitro reports[6]. In addition to having important ramifications as to the use of azithromyin in the setting of post-burn infections, it also relates to the potential for treatment failure in Cystic Fibrosis patients. The combination of azithromycin and tobramycin are used frequently in the setting of a CF pulmonary exacerbation.

Ultimately, we expect that two manuscripts will be generated directly from the findings of this proposal, and that this work will serve as the basis for a R01 research grant submission to the NIH and/or a DMRDP extramural Military Infectious Diseases Clinical Trial Award.

Conclusion

Our findings did not support the central hypothesis of this grant, which was that a dual therapeutic approach of targeted anti-inflammation and a biofilm specific antibiotic will significantly limit severe local and systemic infection associated with serious burns and wounds. We were able to prove that by briefly stopping the influx of neutrophils to the site of thermal injury with the peptide N2, the subsequent wound severity is decreased. This resulted in decreased early *P. aeruginosa* wound infection, but this effect was short-lived and likely not biologically significant. With the addition of azithromycin into this model, we unexpectedly observed an antagonistic effect of the two treatments. However, the early administration of azithromycin did demonstrate a consistent benefit as a single agent, which was increased when combined with the anti-*Pseudomonal* antibiotic ciprofloxacin. The combination of azithromycin with tobramycin proved to be antagonistic.

We believe that the importance and utility of this research to the combat situation is clear, as it is entirely feasible that oral (or IV) azithromycin be administered within the first hours of a severe wound or burn, with the goal of reducing the currently high rate of secondary infection by *P. aeruginosa*. This therapy would not lead to increased antibiotic resistance, as *P. aeruginosa* is not sensitive to azithromycin under conventional antibiotic testing. If a secondary infection develops, our findings indicate that the addition of ciprofloxacin would be of greatest benefit. In a larger sense, we believe that this work relates to a number of other medical conditions, as biofilm-mediated infection by *P. aeruginosa* occurs in highly predictable settings, thus lending it to preventive strategies.

References Cited

1. Church, D., S. Elsayed, O. Reid, B. Winston, and R. Lindsay. 2006. Burn wound infections. *Clin Microbiol Rev* 19(2):403-34.
2. Suber, F., M. C. Carroll, and F. D. Moore, Jr. 2007. Innate response to self-antigen significantly exacerbates burn wound depth. *Proc Natl Acad Sci U S A* 104(10):3973-7.
3. Walker, T. S., G. S. Worthen, K. R. Poch, J. G. Lieber, M. T. Saavedra, K. C. Malcolm, M. B. Fessler, M. L. Vasil, and J. A. Nick. 2005. Enhanced *Pseudomonas aeruginosa* biofilm development mediated by human

neutrophils. *Infect Immun* 73(6):3693-701.
4. Moskowitz, S. M., J. M. Foster, J. Emerson, and J. L. Burns. 2004. Clinically feasible biofilm susceptibility assay for isolates of *Pseudomonas aeruginosa* from patients with cystic fibrosis. *J Clin Microbiol* 42(5):1915-22.
5. Wilkinson, R. A., and J. A. Fishman. 1999. Effect of Thermal Injury with *Pseudomonas aeruginosa* Infection on Pulmonary and Systemic Bacterial Clearance. *J Trauma* 47(5):912.
6. Tre-Hardy, M., C. Nagant, N. El Manssouri, F. Vanderbist, H. Traore, M. Vaneechoutte, and J. P. Dehaye. 2010. Efficacy of the combination of tobramycin and a macrolide in an *in vitro Pseudomonas aeruginosa* mature biofilm model. *Antimicrob Agents Chemother* 54(10):4409-15.

Appendices
None

Supporting Data
None

Acronyms and Symbol Definitions

Azm:	azithromycin
C57BL/6J:	standard laboratory mouse strain
cfu:	colony-forming unit
DNA:	Deoxyribonucleic acid
F-actin:	filamentous actin
IgM:	Immunoglobulin M
i.p.:	intraperitoneal
i.v.:	intravenous
MPO:	myeloperoxidase
N2 peptide:	12 mer peptide sequence LMKNMDPLNDNV with homology to NMHC-II
NMHC-II:	nonmuscle myosin heavy chain II
PA:	*Pseudomonas aeruginosa*
Tob:	tobramycin
tp:	topical